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14. ABSTRACT
Elevated levels of all three naturally occurring polyamines, spermine, spermidine and putrescine, have been found in breast cancer tissues. Polyamine analogues have been shown to inhibit cell growth and in some cases induce apoptosis. My studies have demonstrated the ability of CGC-11144 and other oligoamines to inhibit cell growth in human breast cancer cell lines. These oligamines can also suppress epidermal growth factor receptor (EGFR), human epidermal growth factor receptor-2 (HER2) and estrogen receptor(ER)-alpha protein in multiple human breast cancer cell lines. This suppression is both time and dose dependent. A relationship between oligoamine structure, growth inhibition, and suppression of EGFR and HER2 protein expression seems to exist, with higher nitrogen compounds have the greatest effect. This project will demonstrate that oligoamines are novel anti-HER family agents and oligoamine-induced down regulation of HER family members contributes to their cytotoxicity in human breast cancer cell lines. The completion of this project will also provide valuable information about the potential clinical application of oligoamines

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Polyamines, polyamine analogues, human epidermal growth factor receptor (HER) family, epidermal growth factor receptor (EGFR)

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INTRODUCTION

The polyamines, spermine, spermidine and putrescine, are naturally occurring aliphatic cations that are essential for normal cell growth and differentiation (1,2). A number of studies have shown that polyamines play a key role in carcinogenesis and malignant transformation (3,4), thus making the polyamine pathway a therapeutic target of interest. Increased levels of all three naturally occurring polyamines have been found in many types of cancers, including breast cancer (5). Polyamine analogues have been developed to mimic the three natural polyamines and exploit the self-regulatory properties of polyamines. Treatment of human breast cancer cell lines with polyamine analogues has been shown to inhibit cell growth and in some cases induce apoptosis (6-8). One subset of polyamine analogues are conformationally restricted and long chain analogues named oligoamines (9). Our laboratory has focused on the oligoamine, CGC-11144, because of its effects in human breast cancer cells. Our studies have shown that oligoamines, especially CGC-11144, inhibit growth of human breast cancer cell lines in culture and in mouse xenograft models. Preliminary studies have demonstrated the ability of CGC-11144 to downregulate two members of the human epidermal growth factor receptor (HER) family: epidermal growth factor receptor (EGFR/HER1) and HER2. The overexpression of EGFR and HER2 is usually associated with more aggressive tumors and worse prognosis (10,11). Preliminary studies have have also shown that CGC-11144 inhibits cell growth in human breast cancer cell lines. Thus, the hypothesis underlying this proposal is that oligoamines are novel anti-HER family agents and oligoamine-induced down regulation of HER family members contributes to their cytotoxicity in human breast cancer cell lines. The studies proposed here are designed to elucidate the molecular mechanisms by which polyamine analogues inhibit the expression and activity of the HER family. The results of these experiments will lead to a better understanding of the cytotoxic action of polyamine analogues against human breast cancer and provide valuable information about the potential clinical application of oligoamines.

BODY

Specific Aim 1: To investigate the mechanisms by which oligoamines downregulate EGFR and HER2 expression.

My preliminary studies suggested that the oligoamine, CGC-11144, downregulates the expression of EGFR and HER2 protein in several human breast cancer cell lines as documented by Western blot analysis. These studies were extended to evaluate the time-course and dose-response of human breast cancer cell lines with variable expression patterns for estrogen receptor (ER), EGFR, and HER2 (Figure 1 and Figure 2).

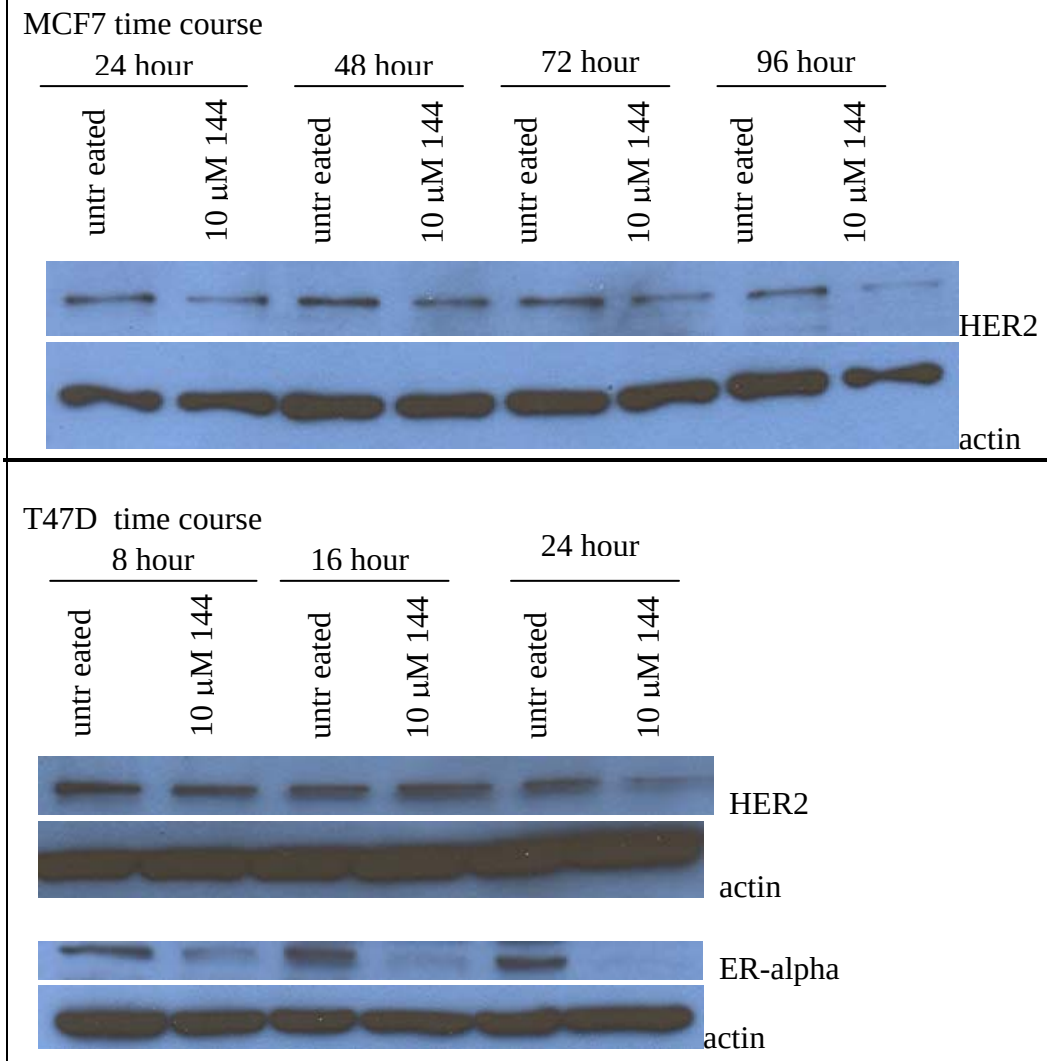

Figure 1. Western blot analysis of MCF7 and T47D breast cancer cell lines treated 10 μM CGC-11144 at multiple time points. Antibodies specific for HER2 (185kDa) and ER-alpha (66kDa) were used. Actin antibody was used as a control to ensure equal loading.

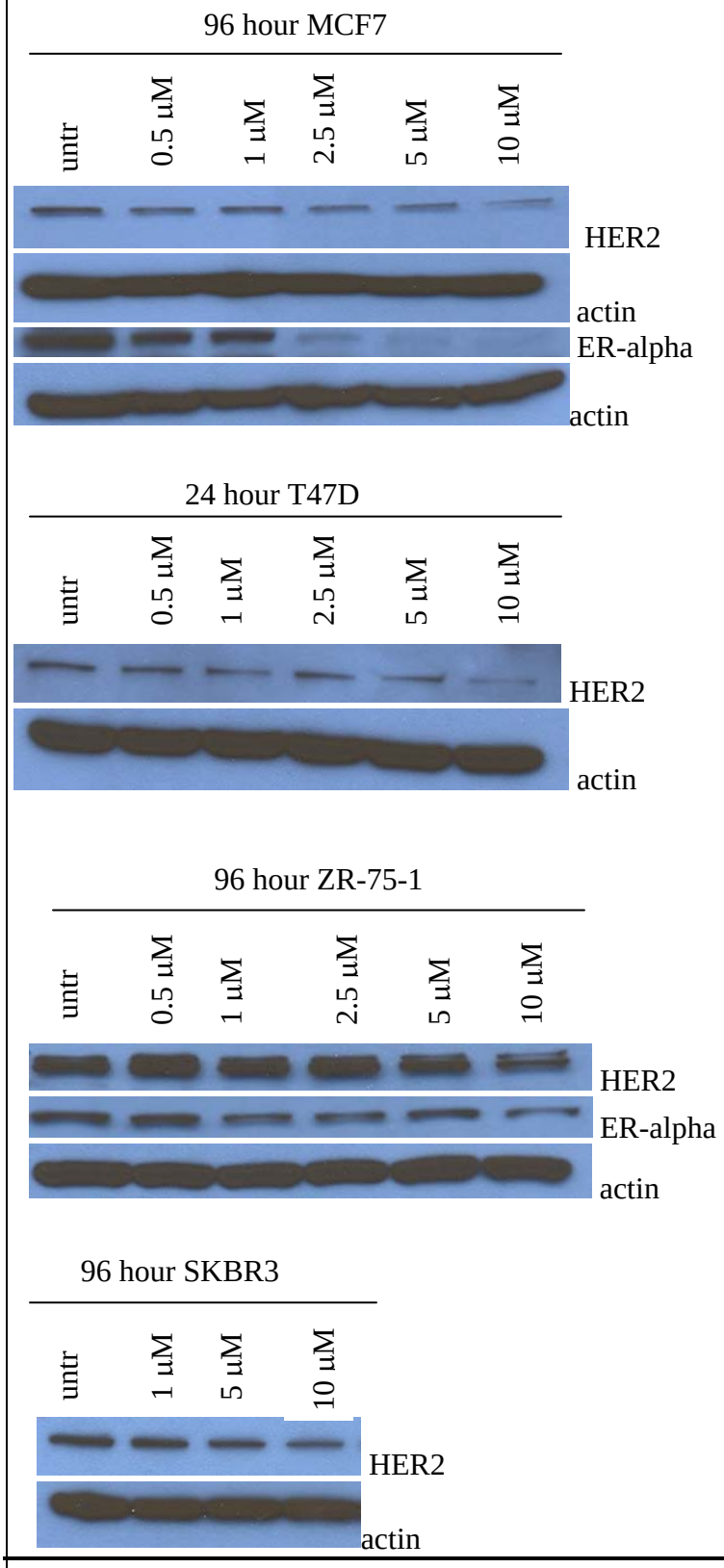

Figure 2: Western blot analysis of MCF7, T47D, ZR-75-1 and SKBR3 breast cancer cell lines treated with CGC-11144 in concentrations ranging from 0-10 µM.Antibodies specific for HER2 (185kDa) and ER-alpha (66kDa) were used. Actin antibody was used as a control to ensure equal loading.

Figures 1 and 2 respectively, demonstrate a time and dose-dependence of HER2 and ER-alpha protein downregulation in multiple cell lines treated with CGC-11144. Studies were expanded to evaluate other oligoamines with varying numbers of nitrogens.

Table 1: Oligoamines and their corresponding number of nitrogens

Oligoamine	Number of nitrogens
CGC-11144	10
CGC-11150	10
CGC-11159	10
CGC-11157	8
CGC-11158	8
CGC-11160	8
CGC-11299	8
CGC-11231	6
CGC-11287	6
CGC-11288	6
CGC-11122	5
CGC-11128	5
CGC-11141	5
CGC-11215	4
CGC-11245	4
CGC-11253	4
CGC-11286	4
CGC-11293	4

The effects of CGC-11144 and other oligoamines on HER2 and ER-alpha protein expression in MCF7 were evaluated with immunoblotting (Figure 3). It is demonstrated that oligoamines, in addition to CGC-11144, have the ability to downregulate HER2 and ER-alpha expression. The extent of this regulation seems to be dependent on the number of nitrogens. The higher the number of nitrogens, the greater the downregulation.

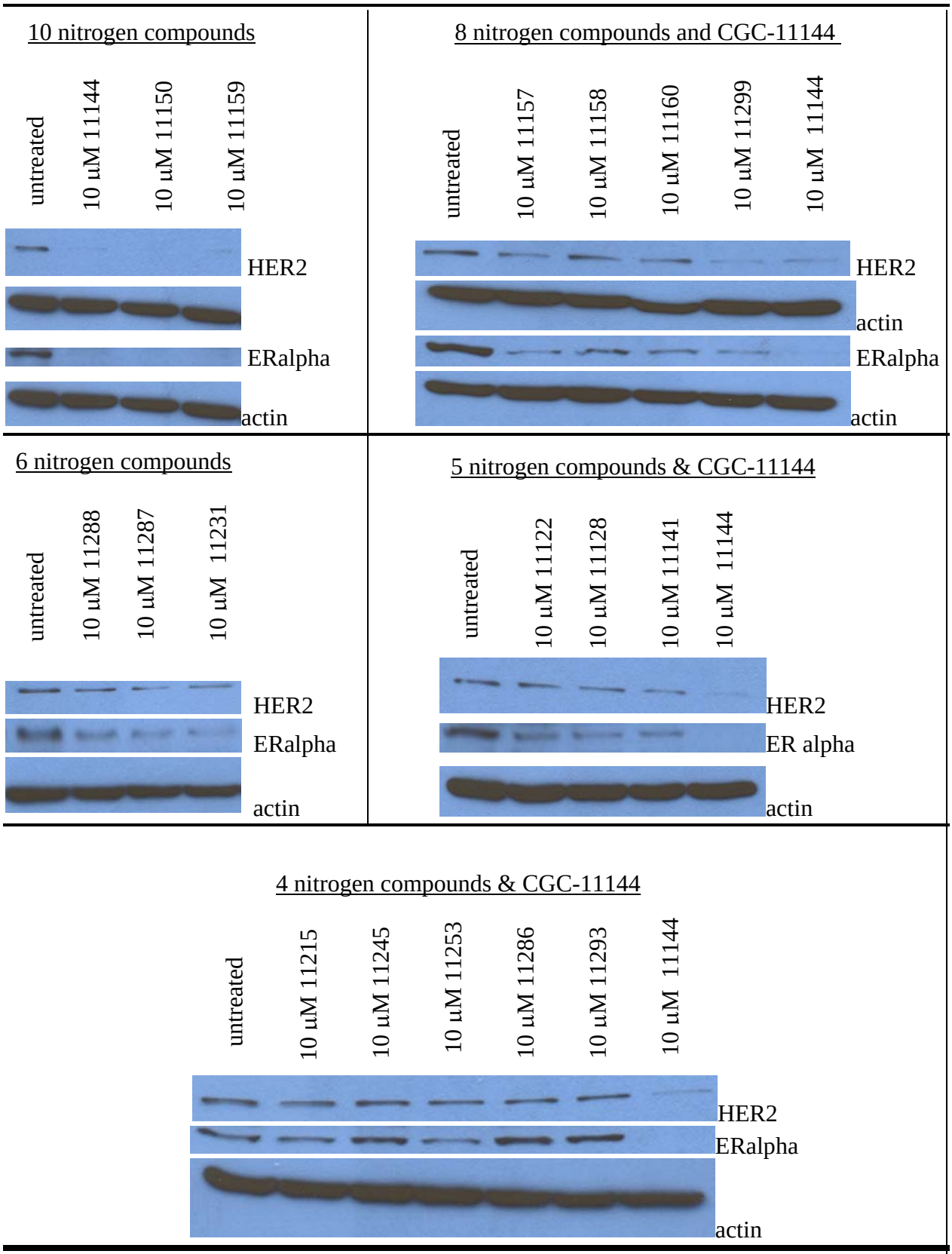

Figure 3: Western blot analysis of MCF7 treated with 10 µM oligamines. Antibodies specific for HER2 (185kDa) and ER-alpha (66kDa) were used. Actin antibody was used as a control to ensure equal loading.

Immunoblotting of breast cancer cell lines T47D and HER2-overexpressing SKBR3 treated with various 8 and 10 nitrogen oligoamines were also used to evaluate HER2 and ER alpha protein expression (Figure 4). The results were similar to MCF7: 10 nitrogen compounds generally had a greater effect on HER2 and ER alpha protein downregulation compared to that of the 8 nitrogen compounds. EGFR protein was examined in SKBR3 and it was shown that both 8 and 10 nitrogen compounds generally decrease EGFR protein below the limits of immunoblotting detection.

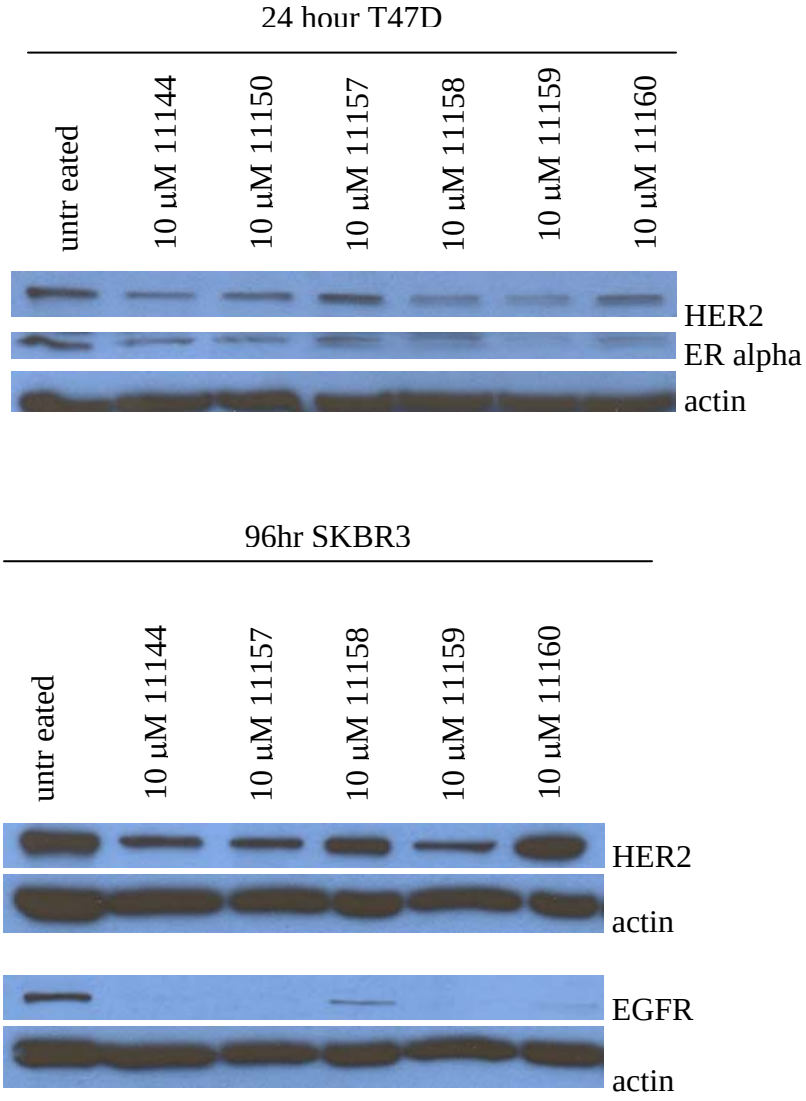

Figure 4: Western blot analysis of T47D and SKBR3 treated with 10 µM oligamines with 8 and 10 nitrogens. Antibodies specific for HER2(185kDa), ERalpha (170kDa) and EGFR (170kDa) were used. Actin antibody was used as a control to ensure equal loading.

In the original proposal my preliminary studies using MTT assays confirmed that CGC-11144 inhibits the growth of several breast cancer cell lines and the IC_{50} values were approximately 0.2-1.0 μM. These studies were extended in MCF7 to include the oligoamines listed in Table 1 with concentrations ranging from 0 to 50 μM. The resulting IC_{50} values were 0.2 - 30 μM, with the higher nitrogen oligamines having lower values.

Table 2:
96 hour MTT Assays - MCF7 treated with various oligoamines

Oligoamine	IC_{50} (μM)
CGC-11144	0.4
CGC-11150	0.2
CGC-11159	0.25
CGC-11157	0.2
CGC-11158	0.3
CGC-11160	0.3
CGC-11299	0.4
CGC-11231	1
CGC-11287	n/a
CGC-11288	3
CGC-11122	1
CGC-11128	2
CGC-11141	n/a
CGC-11215	3
CGC-11245	10
CGC-11253	3
CGC-11286	30
CGC-11293	n/a

The compounds CGC-11287, 11141 and 11293
Preliminary real time-PCR data shows that treatment on HER2 overexpressing SKBR3 with 10 μM CGC-11144 decreases HER2 mRNA (Figure 5). This correlates with the downregulation of HER2 protein shown previously in Figure 2.

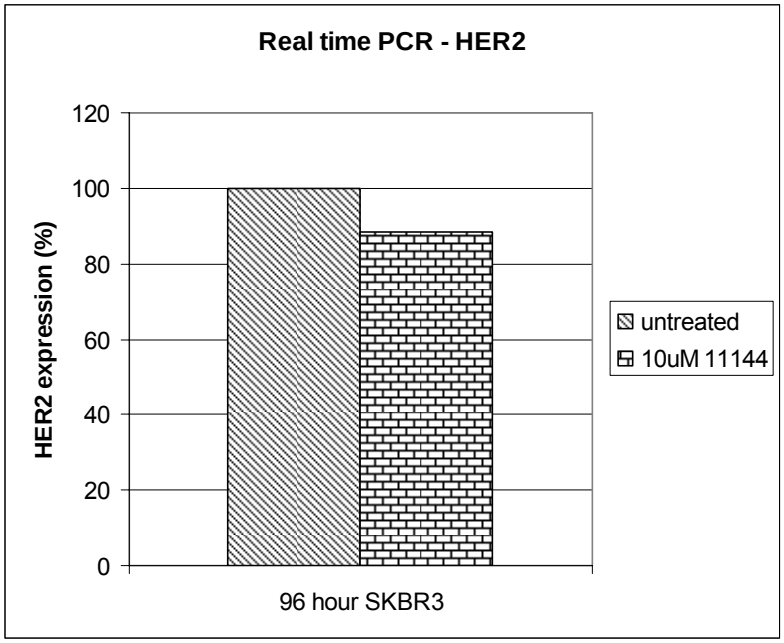

Figure 5: Real time PCR results of SKBR3 treated with 10 μM CGC-11144. RNA was be isolated from oligoamine-treated cells using Trizol method. M-MLV reverse transcriptase was used to generate cDNA, followed by PCR to assess mRNA expression. The levels of mRNA were then quantified using real time PCR.

Real time PCR studies will be continued to access the effects of CGC-11144 and other oligoamines on multiple breast cancer cell lines.

Specific Aim 2: To determine the role of EGFR and HER2 in oligoamine-induced cytotoxicity. Future experiments will address the goals set forth in this aim.

Specific Aim 3: To determine if oligoamines can overcome endocrine resistance. Future experiments will address the goals set forth in this aim.

KEY RESEARCH ACCOMPLISHMENTS

- CGC-11144 and other oligoamines inhibit growth and suppress HER2, EGFR and ER-alpha protein expression in multiple breast cancer cell lines.
- There is a time and dose-dependent relationship between oligoamines and HER2 and ER-alpha protein downregulation in multiple breast cancer cell lines.
- There is a relationship between oligoamine structure and both growth inhibition and HER2, EGFR and ER-alpha protein expression in multiple breast cancer cell lines.
- Preliminary real time PCR data in SKBR3 suggest suppression of HER2 mRNA levels.

REPORTABLE OUTCOMES

Presentations:

Polyamine Analogues as Novel Anti-HER Family Agents in Human breast Cancer. Richards T. Cellular and Molecular Medicine Program Fall Retreat, Johns Hopkins University, September 2006.

CONCLUSIONS

CGC-11144 and other oligoamines have the ability to inhibit growth and suppress HER2, ER-alpha and EGFR protein expression. This suppression is both time and dose dependent. There is a relationship between oligoamine structure, growth inhibition, and suppression of EGFR and HER2 protein dowregulation, with higher nitrogen compounds have the greatest effect. In addition to CGC-11144, future work will focus on higher nitrogen oligoamines. The completion of specific aim 1 and the initiation of specific aim 2 and 3 will lead to a better understanding of the cytotoxic action of polyamine analogues against human breast cancer and provide valuable information about the potential clinical application of oligoamines.

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